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## ORIGINAL ARTICLE

# Sensory evaluation and chemical analysis of exhaled and dermally emitted bioeffluents

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**Abstract**

Conditions in which exhaled and dermally emitted bioeffluents could be sampled separately or together (whole-body emission) were created. Five lightly dressed males exhaled the air through a mask to another, identical chamber or without a mask to the chamber in which they were sitting; the outdoor air supply rate was the same in both chambers. The carbon dioxide concentration in the chamber with exhaled air was 2000 ppm. Chamber temperatures were 23°C or 28°C, and ozone was present or absent in the supply airflow. When dermally emitted bioeffluents were present, the perceived air quality (PAQ) was less acceptable, and the odor intensity was higher than when only exhaled bioeffluents were present. The presence or absence of exhaled bioeffluents in the unoccupied chamber made no significant difference to sensory assessments. At 28°C and with ozone present, the odor intensity increased and the PAQ was less acceptable in the chambers with whole-body bioeffluents. The concentrations of nonanal, decanal, geranylacetone, and 6-MHO were higher when dermally emitted bioeffluents were present; they increased further when ozone was present. The concentration of squalene then decreased and increased again at 28°C. Dermally emitted bioeffluents seem to play a major role in the sensory nuisance experienced when occupied volumes are inadequately ventilated.

**KEYWORDS**

chemical analyses, dermally emitted bioeffluents, exhaled bioeffluents, human bioeffluents, indoor air quality (IAQ), sensory assessments

## 1 | INTRODUCTION

### 1.1 | Background

Body odor originates from sweat and sebaceous secretions from skin, and from foul breath. The latter includes the pollutants emitted when breathing, including gases from the digestive tract. Body odor includes intestinal gases (flatulence). Although considered generally as nontoxic, body odor may evoke a feeling of nausea and even reduced appetite in some people.<sup>1</sup> Recently published work shows that exposure to emissions from humans (human bioeffluents) can increase sleepiness, fatigue, headaches, and difficulty in concentrating<sup>2–4</sup> and reduce cognitive performance, including the ability to take decisions.<sup>5</sup> There is substantial evidence on how body odor is perceived by humans.<sup>6–9</sup> This published research provided the basis for contemporary ventilation standards.<sup>10,11</sup>

The connection between body odor and ventilation goes back to the 19th century when Pettenkofer<sup>12</sup> associated discomfort with emissions from humans, rejecting the earlier theories that associated discomfort with the presence of carbon dioxide (CO<sub>2</sub>) or lack of oxygen (O<sub>2</sub>). Pettenkofer proposed the use of CO<sub>2</sub>, the main human inorganic bioeffluent, as a marker of the quality of air polluted by human bioeffluents and as an indicator of ventilation efficiency in the presence of humans. He postulated that the sources producing other indoor pollutants not emitted by humans should be first eliminated. He then proposed that a CO<sub>2</sub> concentration of 1000 ppm could be considered the hygienic limit, above which indoor air quality is unacceptable. He assumed 500 ppm as the concentration of outdoor CO<sub>2</sub>, which is much higher than the current ambient level, slightly above 400 ppm. CO<sub>2</sub> has subsequently been used universally as a proxy for

indoor air quality. Pure CO<sub>2</sub> at the levels normally occurring indoors was not considered by Pettenkofer to constitute a risk for occupants. Recent studies by Zhang et al<sup>3,4</sup> and Liu et al<sup>13</sup> show that pure CO<sub>2</sub> below 5000 ppm does not produce sensory discomfort, increase self-reported acute health symptoms, or reduce the performance of simple cognitive tasks, thus confirming Pettenkofer's assumption. Two other recent experiments suggest however that pure CO<sub>2</sub> at concentrations as low as 1000 to 2500 ppm can reduce the ability to take decisions in a stressful situation.<sup>14,15</sup> One experiment reported that a pure CO<sub>2</sub> concentration of 4000 ppm reduced perceived air quality (PAQ) and increased acute health symptoms, and that the performance of a proofreading task which can be considered a typical office task was reduced in one of the two series of experiments that were reported.<sup>16</sup> The validity and proposed mechanisms of these conflicting findings are still to be confirmed.

Humans emit many different volatile organic compounds (VOCs). Recently, Liu et al<sup>17</sup> reported that the compounds associated with the presence of humans contribute up to 40% of the measured daytime VOC concentration in indoor spaces. In another recent study, Tang et al<sup>18</sup> reported that human-emitted VOCs were the dominant source during occupied periods in a well-ventilated classroom (57%) together with ventilation supply air, which was the second most important source of pollution (35%). The types of pollutants emitted depend on the nutrition and hygiene standards of the occupants, their health condition, and even their addictions, such as alcohol or cigarette smoking.<sup>19</sup> Furthermore, even changes in metabolism affect VOC breath e.g.,<sup>20</sup> and sweat composition e.g.,<sup>21</sup> Some studies measured whole-body bioeffluents, e.g.,<sup>22–25</sup> others analyzed bioeffluents emitted when breathing (exhaled bioeffluents) and through skin (dermally emitted bioeffluents) or bioeffluents from a particular part of the body, for example the oral cavity, e.g.,<sup>26–30</sup> skin excluding the head, e.g.,<sup>31–35</sup> axillae, e.g.,<sup>36–45</sup> scalp, e.g.,<sup>46–48</sup> hand, e.g.,<sup>34,46,48–58</sup> feet, e.g.,<sup>48,59,60</sup> and intestines, e.g.,<sup>61,62</sup>

There is consequently a fairly large body of literature showing what types of pollutants are emitted by different body parts and what factors influence the emission rates. This information is described briefly in the following and summarized in Table 1. Among the research reported there are however no studies that examined the effects of bioeffluents emitted by different body parts on the quality of air as it is perceived by humans. In particular, there are no studies that examined whether exhaled and dermally emitted bioeffluents produce different sensory perceptions or whether they contribute equally to the sensory nuisance associated with the body odor. The present work was consequently undertaken to fill this gap in knowledge. It was additionally examined whether the presence or absence of any specific compounds emitted by humans could contribute to the above differences.

## 1.2 | Summary of previous measurements of human bioeffluents

Krotoszynski and Dravnieks<sup>22</sup> sampled vapors from the whole body by placing subjects on a Teflon-lined stretcher in a glass tube. Five compounds were found to be common to white, Afro-American and

### Practical Implications

- The present results suggest that ozone should be avoided in occupied volumes and that high temperatures may create conditions that lead to increased emission of bioeffluents and so should also be avoided. The latter finding implies that the ventilation rate required to control emissions from humans should depend not only on the number of occupants but also on the air temperature. The present results form a basis for developing new technologies and solutions for the source control of human bioeffluents, such as absorptive clothing or personalized ventilation.

Indian males and to white females. These were acetone, butanol, ethanol, lactic acid, and pyruvic acid. Ellin<sup>23</sup> sampled compounds in the headspace surrounding a person. Forty-six seminude males were placed, one at a time, in a sealed chamber made of glass, stainless steel, and teflon. Around 330 compounds were detected and 135 compounds were identified. The five compounds with the highest concentration, which were emitted by all subjects, were acetone, butanol, ethanol, isoprene, and toluene. Wang<sup>24</sup> investigated bioeffluents in an auditorium. Samples were taken from the inlet and exhaust of the air conditioning system. Sixteen compounds were considered as bioeffluents, and acetone, acetic acid, butyric acid, ethanol, and methanol, were found in high concentrations. The main compounds measured in bedrooms and associated with the presence of human bioeffluents are according to Hanihara et al<sup>25</sup> C2-10 fatty acid, C6-10 aldehydes, 6-methyl-5-hepten-2-one (6-MHO), (E)-6,10-dimethyl-5,9-undecadien-2-one (geranylacetone), and 2-ethyl-hexanol.

VOCs in human breath were identified by Chen et al<sup>26</sup> who concluded that the intensity of the odor is directly related to the amount of dimethyl sulfide in the breath. At that time (1970), oral bioeffluents were used as a non-invasive indicator of health. In the study of Sun et al<sup>27</sup> the air exhaled by 111 subjects was sampled using a specially developed experimental system, and 645 VOCs were detected; on average, over fifty different types of VOCs from each subject were detected. In a summary of studies measuring human bioeffluents, Bluysen<sup>28</sup> and Tonzetich<sup>29</sup> reported that hydrogen sulfide, methyl mercaptan, and dimethyl sulfide were believed to be the main sources of poor air quality caused by human exhalation. Fenske and Paulson<sup>30</sup> reported that the major VOCs in the exhaled breath of healthy individuals were isoprene, acetone, ethanol, methanol, and other alcohols; minor components included pentane and higher aldehydes and ketones.

Zhang et al<sup>31</sup> measured emissions from 30 healthy subjects wearing gas masks in a sealed chamber with almost no ventilation. Organic pollutants emitted from the skin were identified. In all, 893 VOCs were detected, an average of 71 VOCs (SD=21.2, range=19-101) from each subject. Logan et al<sup>32</sup> analyzed the organic pollutants emitted by human volunteers, who were placed in

**TABLE 1** Summary of previous studies measuring volatile organic pollutants emitted by humans; the numbers shown in the table relate to studies itemized in the reference list<sup>a,b</sup>

Compound	Odor threshold (Nagata, 2003)	Odor threshold (Devos et al, 1990)	Measurements of whole-body bioeffluents (dermally emitted and exhaled)	Measurements of exhaled bioeffluents	Measurements of dermally emitted bioeffluents				Measurements of bioeffluents from intestines
					Skin except head	Axillae	Scalp	Hand, forearm	Feet
Acetic acid	16 µg/m <sup>3</sup>	360 µg/m <sup>3</sup>	24						
Acetone (2-propanone)	110 mg/m <sup>3</sup>	35 mg/m <sup>3</sup>	22, 23, 24	29					
Ammonia	1.1 mg/m <sup>3</sup>	4.1 mg/m <sup>3</sup>							62
Androstanol	-	-				37			
Androstenone	-	-				38			
Butanol	0.1 mg/m <sup>3</sup>	1.5 mg/m <sup>3</sup>	22, 23						
Butyric acid	0.8 µg/m <sup>3</sup>	15 µg/m <sup>3</sup>	24						
Decanal	2.8 µg/m <sup>3</sup>	5.9 µg/m <sup>3</sup>			32, 33, 34			49, 53, 56	
Dimethyl hexanedioate	-	-						56	
Dimethyl sulfide	8.3 µg/m <sup>3</sup>	5.9 µg/m <sup>3</sup>		28, 29					
Ethanol	1.1 mg/m <sup>3</sup>	55 mg/m <sup>3</sup>	22, 23, 24	30			48		
γ-decalactone	-	-							
Geranylacetone	-	-			32, 33, 34			49	
Heptanal	-	23 µg/m <sup>3</sup>			33				
Hydrogen sulfide	0.6 µg/m <sup>3</sup>	26 µg/m <sup>3</sup>		28, 29					60, 61
Isoprene	150 µg/m <sup>3</sup>		23	30					64
Isovaleric acid	0.4 µg/m <sup>3</sup>	11 µg/m <sup>3</sup>				40, 41, 42			48, 61
Lactic acid	-	-	22						
Lilial	-	-						49	
Methanethiol	-	2.0 µg/m <sup>3</sup>							60
Methanol	47 mg/m <sup>3</sup>	190 mg/m <sup>3</sup>	24	30					
Methylmercaptan	0.2 µg/m <sup>3</sup>	-		28, 29					
Nonanal	2.2 µg/m <sup>3</sup>	14 µg/m <sup>3</sup>			32, 33, 34			49, 53, 56	
Octanal	0.1 µg/m <sup>3</sup>	7.2 µg/m <sup>3</sup>			32, 33				
Octanoic acid	-	24 µg/m <sup>3</sup>						53	
Pentadecane	-	-						53	
Phenol	24 µg/m <sup>3</sup>	430 µg/m <sup>3</sup>						56	
Pyruvic acid	-	-	22						
Toluene	1.4 mg/m <sup>3</sup>	5.9 mg/m <sup>3</sup>	23						
2-furancarboxaldehyde	-	-						56	

(Continues)

TABLE 1 (Continued)

Compound	Odor threshold (Nagata, 2003)	Odor threshold (Devos et al, 1990)	Measurements of whole-body bioeffluents (dermally emitted and exhaled)	Measurements of exhaled bioeffluents	Measurements of dermally emitted bioeffluents				Measurements of bioeffluents from intestines
					Skin except head	Axillae	Scalp	Hand, forearm	Feet
2-furanmethanol	-	-	-	-	-	-	-	-	56
3-hydroxy-3-methylhexanoic acid	-	-	-	-	-	44, 45	-	-	-
3-methyl-2-hexenoic acid	-	-	-	-	-	44, 45	-	-	-
6-MHO	-	250 µg/m <sup>3</sup>	-	-	32, 33, 34	-	-	-	49

<sup>a</sup>Some volatile products of ozone/skin oil chemistry are not presented.

<sup>b</sup>It should be noted that besides other factors mentioned in the text, the different sampling procedures and their limitations could contribute to differences in measured bioeffluents among the reported studies.

individual plastic bags lined with aluminum with only their heads outside the bag. They identified 24 VOCs that were additionally shown to attract *Aedes* mosquitoes. The dermally emitted bioeffluents that were found to play a key role in attracting insects were 6-MHO, decanal, nonanal, octanal, and geranylacetone. Dormont et al<sup>33</sup> noted that only a few families of compounds are represented among dermal emissions and that these include the compounds such as carboxylic acids of various chain lengths and derivative esters, aldehydes, alkanes, short chain alcohols, and some ketones. Harraca et al<sup>34</sup> collected dermally emitted bioeffluents by placing volunteers in customized heat-sealed oven bags; their heads were kept outside the bag as in the studies reported by Logan et al<sup>32</sup> Six main compounds were observed, including 6-MHO, decanal, geranylacetone, heptanal, nonanal, and octanal. Gallagher et al<sup>35</sup> reported that the dermally emitted amounts of some compounds can vary with age. Three compounds were found to be biomarkers of increased age: dimethylsulphone, benzothiazole, and nonanal. No significant differences related to age or locus were found for octanal or decanal.

The axillary region is a particularly important source of diverse VOCs.<sup>36</sup> The source strength in this region is a result of interactions between secretions of eccrine, sebaceous, and apocrine glands and the resident bacteria. Compounds contributing to the profile of the air quality of air from the axillary region include androstenol, which has a musky smell,<sup>37</sup> androstenone, which is a ketone with a urine-like smell<sup>38</sup> and isovaleric acid.<sup>39</sup> In particular, *lipophilic corynebacteria*, which dominate the commensal bacterial community in the axillary region of the skin, are largely responsible for the production of malodorous volatile products.<sup>40-42</sup> There are also other often commensal bacteria (e.g., *Staphylococcus epidermidis*) that are responsible for the smell from axilla.<sup>43</sup> In some studies, volatile profiles have been reported to be dominated by two key odoriferous compounds, 3-methyl-2-hexenoic acid and 3-hydroxy-3-methylhexanoic acid.<sup>44,45</sup>

The scalp is rich in lipids, owing to the high density of sebaceous glands.<sup>46</sup> The source strength of the scalp is a result of the *propionibacterium acnes* in the hair follicles. The major scalp population is the yeast *pityrosporum ovale*, which metabolises lipid substances to fatty acids, and glycerol, that both undergo ring closure to the volatile and odorous  $\gamma$ -lactones.<sup>47</sup> Labows<sup>48</sup> indicated that  $\gamma$ -decalactone could be responsible for the smell of unwashed hair.

Odors from the hands have been largely investigated in the context of forensic science.<sup>48-50</sup> Hundreds of VOCs emanating from palms were found, for example, aromatics, amides, amines, halides, sulfides, and sulfonyls.<sup>51,52</sup> The profiles of VOCs emitted by the hands are often dominated by aldehydes and ketones, and particularly by 6-MHO, decanal, geranylacetone, nonanal, and undecanal.<sup>46,49</sup> The same compounds have also been regularly found to be the major compounds emitted from the forearm, together with some alkanes and carboxylic acids.<sup>34,53,54</sup> Comparing hand odors from 10 subjects, Curran et al<sup>55</sup> identified 24 main compounds that can be considered to be a part of the "primary odor" profile of human scent. Six compounds were found to be highly frequent among the emissions from hands and they include 2-furancarboxaldehyde, 2-furanmethanol, decanal, dimethyl

hexanedioate, nonanal, and phenol. Among them, decanal, nonanal, and some carboxylic acid-methyl esters have been isolated regularly from hand emissions in other studies.<sup>49,51,56–58</sup>

*Brevibacterium epidermidis* (*B. epidermidis*) is resident on human skin, especially in areas such as toeweb. This organism produces methanethiol gas, which accounts for a large part of the characteristically cheesy smell from unwashed feet.<sup>59</sup> Other gaseous substances, for example hydrogen sulfide<sup>63</sup> and isovaleric acid,<sup>48</sup> also contribute to the source strength of the feet. Ara et al<sup>60</sup> reported several short chain fatty acids in solvent extracts of foot sweat and considered that isovaleric acid was most likely responsible for strong foot odor.

Intestinal gas causing flatulence contains a wide variety of gases, for example ammonia, hydrogen sulfide, volatile amino acids, and short chain fatty acids are also emitted from the intestines in trace concentrations of <1% of all intestinal gases but can easily be detected by humans.<sup>61</sup> Five major compounds that represent 99% of bowel gas are N<sub>2</sub>, O<sub>2</sub>, CO<sub>2</sub>, H<sub>2</sub>, and CH<sub>4</sub> though these do not necessarily lead to odor nuisance. Garner et al<sup>64</sup> found 101 VOCs emitted from fecal gas of healthy individuals out of which 44 VOCs were common for all individuals. Kirk<sup>62</sup> sampled excreted intestinal gases (flatus) from 45 normal subjects; it was excreted at an average rate of 1.47 mL per minute after an ordinary diet, and found an average concentration of hydrogen sulfide (H<sub>2</sub>S) of 0.00028%.

In addition to the emissions that result from the physiological processes that occur in the human body, recent studies show that many compounds can be created when compounds emitted by humans, mainly constituents of skin oils, participate in reactions with ozone. These reactions occur in the presence of humans and also in spaces that were previously occupied by humans, due to shedding of the skin and the soiling of surfaces with human skin flakes containing skin oils. The major reaction that occurs is between ozone and squalene, while acetone, geranylacetone, 6-MHO, decanal, and nonanal are the major products.<sup>65–67</sup> Squalene and geranylacetone are the major primary precursors for acetone and 6-MHO, and acetone may also be formed by 2-methyl-2-docosene. Yang et al<sup>67</sup> showed that the possible primary precursors of four major products could be the compounds contained in the skin oil deposited in clothing. The formation of nonanal can be mainly attributed to reactions between ozone and unsaturated fatty acids such as 9-octadecenoic acid and (Z)-7-hexadecenoic acid and their derivatives, such as (Z)-13-docosenamide. 6-hexadecenoic acid (the most abundant unsaturated fatty acid of skin-oiled clothing) and 8-octadecenoic acid are the main reagents that contribute to the generation of decanal. Wax esters and triglycerides have a structure that is similar to that of the above-mentioned fatty acids and can also contribute to the formation of nonanal and decanal. Schwarz et al<sup>68</sup> reported that formaldehyde can be detected in human breath and its emissions can be accelerated during reactions between squalene and ozone. Liu et al<sup>17</sup> showed that phenol is one of the human bioeffluents that can be found in exhaled breath and is a product of surface oxidation of human skin lipids. Liu et al<sup>17</sup> concluded that VOCs produced from ozonolysis of human skin lipids were positively correlated with the concentration of CO<sub>2</sub> and negatively with the concentration of O<sub>3</sub>.

These results show that ozonolysis of skin lipids takes place indoors in buildings and that humans are an important source of indoor VOCs and a sink for indoor O<sub>3</sub>.

Occupants can also be a source of the constituents of bathing soaps, shampoos, lotions, deodorants, perfumes, and other cosmetic products including paper towels as a result of their hygienic routines, even if these are conducted elsewhere.<sup>69</sup> Consumer products can include monoterpenes, linalool, and cyclic volatile siloxanes, the latter of which are added to antiperspirants and have been reported as dominating VOC emissions from humans in an occupied classroom.<sup>18</sup> These compounds can vary greatly between individuals depending on their cosmetic preferences and hygienic standards and are associated with their behavior and preferences rather than with their physiology.

### 1.3 | Objective

The main objective of this research was to compare the sensory effects produced by exhaled bioeffluents with those produced by dermally emitted bioeffluents and compare them with the sensory effects produced by whole-body bioeffluents, that is, a combination of the two. A chemical analysis of bioeffluents was performed to examine whether any observed sensory effects can be attributed to the presence of specific pollutants.

## 2 | METHODS

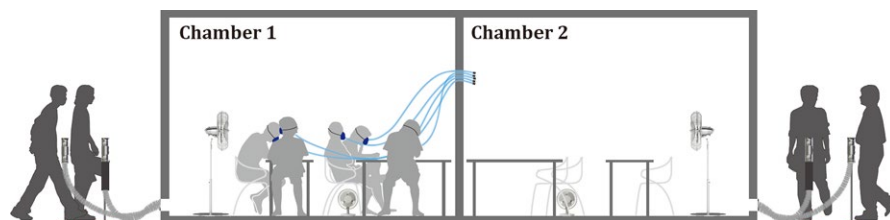
### 2.1 | Facilities

The experiments were performed in the twin stainless steel chambers at Technical University of Denmark that were described in detail by Albrechtsen.<sup>70</sup> Each chamber had a volume of 22.5 m<sup>3</sup> (floor area of 9 m<sup>2</sup> × 2.5 m height) but with recirculation ducts had a volume of 30 m<sup>3</sup>. The air in each chamber was recirculated during experiments to ensure proper mixing. Additionally, a desktop fan and a standing fan were in operation. Both chambers were furnished only with the stainless steel chairs and tables used by the subjects. The outdoor air change rates were maintained in both chambers at 1.5 h<sup>-1</sup>. The pollution from outdoor air, if any, is expected to have contributed equally to the pollution level in both chambers. The air change rate was measured using a constant dosing tracer gas (CO<sub>2</sub>) technique and an Innova 1302 gas analyzer. The outdoor air supply rate was selected so that the CO<sub>2</sub> concentration with five persons sitting in the chamber would reach 2000 ppm.

The principal elements of the experimental set-up in the twin chambers are presented in Figure 1. They are described in detail in the text that follows.

### 2.2 | Subjects

Five males were recruited to sit in one chamber as a source of bioeffluents. They were all Caucasian, non-smokers, and had no chronic diseases. Twenty-three additional subjects were recruited to perform sensory evaluations. Table 2 summarizes information about the source



**FIGURE 1** Principal elements of the experimental set-up in the twin chambers. The figure shows subjects outside the chambers (performing the sensory assessments) and the subjects inside the chambers (a source of bioeffluents)

**TABLE 2** Demographic data of recruited subjects

Characteristic description	Subjects sitting in the chamber	Subjects performing sensory evaluations
Total	5	23
Gender: males, females	5, 0	12, 11
Age (mean±SD) years old	24.4±2.0	24.5±3.0
Height (mean±SD) cm	174.6±4.8	-
Weight (mean±SD) kg	73.4±3.4	-
Occupation: students, faculty	5, 0	22, 1
Smokers	0	1
Subjects reporting they had any allergy including hay fever	0	1
Subjects reporting they had asthma	0	0
Subjects reporting they had any chronic disease	0	1 (Narcolepsy)
Subjects considering themselves more sensitive to odorous/pungent substances	4	16
Subjects reporting they adapted easily to most odorous/pungent substances	4	17
Subjects reporting they were easily alerted by odorous/pungent substances	4	17

subjects and the subjects who performed the sensory evaluations. This information was provided by the subjects themselves. No one was examined medically, and no tests were performed to examine the ability of the subjects to perceive odors. Subjects with impaired hearing and those indicating that they considered themselves unable to discriminate odors or distinguish different intensities of odors were eliminated during recruitment. The majority of subjects were students. The subjects were compensated financially for their participation in the experiments.

## 2.3 | Experimental conditions and procedures

Twelve experimental conditions (of which nine were with the human bioeffluents) were created by combining the following conditions (Table 3): chamber with dermally emitted bioeffluents, chamber

with exhaled bioeffluents, chamber with whole-body (dermally emitted+exhaled) bioeffluents, the supply air with ozone naturally present or eliminated, and the chamber temperature set at 23°C or 28°C.

Five lightly dressed male subjects occupied one chamber as the source of bioeffluents. They were instructed not to drink alcohol or eat spicy food or garlic on both the day prior to and the day of the experiment. They received fragrance-free soap and shampoo to be used in the evening prior to each experiment instead of their usual hygienic products. They were additionally instructed not to use strong deodorants, perfumes, or antiperspirants. The subjects wore sleeveless T-shirts, half pants or trunks, and socks. The estimated clothing insulation was 0.20 clo. The T-shirts were provided by the experimental team; they were bought brand new and washed once with the odor-free detergent. Each time, the new T-shirt was worn. Other clothing was provided by the subjects themselves; this clothing was also washed prior to the experiments with the same odor-free detergent as used to wash the T-shirts. Consequently, if clothing contributed to the pollution load in the chamber, it can be assumed that the contribution was approximately the same in each condition.

To isolate dermally emitted and exhaled bioeffluents, these bioeffluent source subjects sat in one chamber and exhaled the air to the other chamber through breathing masks (Sperian ValuAir Plus 6100V series RP155). The masks were attached to Teleflex medical tubes made of clear vinyl with a diameter of 22 mm that ran through a polyurethane plate separating the twin chambers. The tube length was determined by the sitting position of subjects; two tubes were about 2.0 m in length and three tubes were about 2.5 m. The plate replaced the door separating the chambers. A miniature fan was mounted on the other end of each tube. It operated at a relatively low speed to facilitate the movement of exhaled air through the tube and ensure that all of it entered the second chamber (Figure 1). This was checked and verified by tracer gas measurements. The subjects were instructed to breathe normally through their masks and avoid taking deep breaths or exhaling rapidly so that all the air that was exhaled could be drawn through into the second chamber. They breathed normally without the mask in the whole-body bioeffluent condition. The subjects breathed in the air in the chamber that they occupied, regardless of whether they were wearing the mask.

To remove ozone from the supply air, charcoal filters were installed. In the "ozone present" condition, these charcoal filters were removed, and the ozone concentration in the supply duct was then the result of the ozone occurring naturally outdoors less any ozone scavenging taking place in the duct itself; no ozone generators were used to increase the ozone concentration.



**TABLE 3** Measured air temperature ( $T_a$ ), relative humidity (RH), carbon dioxide concentration ( $\text{CO}_2$ ), and ozone concentration ( $\text{O}_3$ ) in the 12 conditions created in the chambers and investigated in the present experiments; the numbers show average and standard deviation (in parentheses). For ozone, the number in parentheses indicates the outdoor ambient ozone concentration

Conditions in the chambers	23°C with ozone present					23°C with ozone absent					28°C with ozone absent				
	$T_a$ (°C)	RH (%)	$\text{CO}_2$ (ppm)	$\text{O}_3$ (ppb)	$T_a$ (°C)	RH (%)	$\text{CO}_2$ (ppm)	$\text{O}_3$ (ppb)	$T_a$ (°C)	RH (%)	$\text{CO}_2$ (ppm)	$\text{O}_3$ (ppb)	$T_a$ (°C)	RH (%)	$\text{CO}_2$ (ppm)
No bioeffluents present (empty chambers)	22.8 (0.2)	39 (2)	558 (2)	7.4 (16.0)	22.5 (0.4)	44 (6)	548 (22)	N/A (26.0)	28.2 (0.2)	30 (1)	504 (58)	N/A (31.9)	28.2 (0.2)	30 (1)	504 (58)
Exhaled bioeffluents (chamber with the air exhaled through masks)	24.0 (0.5)	34 (1)	1862 (8)	16.6 (24.4)	22.8 (0.1)	54 (5)	1763 (69)	0 (21.6)	27.7 (0.2)	32 (2)	1787 (179)	N/A (28.9)	27.7 (0.2)	32 (2)	1787 (179)
Dermally emitted bioeffluents (chambers with the pollutants emitted through skin when individuals were breathing into the masks)	24.4 (0.1)	33 (0)	533 (2)	13.7 (24.4)	24.8 (0.1)	36 (0)	887 (22)	3.1 (21.6)	27.6 (0.4)	38 (4)	641 (49)	N/A (28.9)	27.6 (0.4)	38 (4)	641 (49)
Whole-body (dermally emitted+exhaled) bioeffluents (emitted by individuals sitting and breathing normally in the chambers without masks)	22.7 (0.1)	43 (3)	2249 (31)	2.2 (16.0)	24.0 (0.1)	53 (1)	1960 (15)	N/A (28.0)	27.9 (0.2)	30 (0)	1880 (27)	4.8 (32.2)	27.9 (0.2)	30 (0)	1880 (27)

The two temperature conditions were maintained by the chamber ventilation system. Relative humidity was not controlled but was measured.

To reduce the time taken to reach steady-state conditions in the chambers in the “ozone absent” conditions, once the subjects entered the chamber, the fans supplying outdoor air to the chambers were turned off. They were then kept off until the  $\text{CO}_2$  concentration had reached 2000 ppm. During this time, the recirculation and mixing fans were on. Upon reaching 2000 ppm, the supply fans were turned back on. In the “ozone present” conditions, the fans supplying outdoor air to the chambers were operated continuously to ensure that ozone was continuously supplied to the chambers. Sensory assessments and chemical measurements began a few minutes after a planned steady-state concentration of  $\text{CO}_2$  in the chambers.

Experiments were carried out on four days in June 2016, each day lasting 180–205 min. On two experimental days, conditions with ozone present in the supply ducts were examined. The chambers were set to 23°C on both days. On these days, the subjects entered the chamber and stayed there for 180 min. The following conditions were created on the first day: chamber without bioeffluents present (empty chambers) and chamber with whole-body (dermally emitted+exhaled) bioeffluents. On the second day, the following conditions were created: chamber with dermally emitted bioeffluents only and chamber with exhaled bioeffluents only. On the two subsequent days, a charcoal filter was installed to remove ozone from the supply air. The chambers were set at 23°C on one day and at 28°C on another day. Each day, the following conditions were created: chamber without bioeffluents present (empty chamber); chamber with exhaled bioeffluents only; chamber with dermally emitted bioeffluents only; and chamber with whole-body (dermally emitted+exhaled) bioeffluents. The order of the conditions was randomized to make it possible to analyze the results for a potential chamber effect. The details of the experimental procedures are given in Appendix S1.

## 2.4 | Measurements

Air temperature, relative humidity, and  $\text{CO}_2$  concentration were measured at two locations inside each chamber. These parameters were recorded every 10 or 30 s by calibrated sensors and were logged. One measurement location was close to the sampling point of air collected for chemical measurements (but not too close to avoid any possible interference), and the other was placed on the chamber wall, half-way up the chamber height.  $\text{CO}_2$  concentrations measured at the two locations were similar, indicating that the air in the chambers was well mixed throughout the entire volume. Ozone was measured with a Model 205 Dual Beam Ozone Monitor (2B Technologies) in the duct containing the air in the chamber. Outdoor ozone concentration was obtained from the nearest monitoring station that was located about 25 km from the university campus; the station was located in a rural area. The accuracy of measuring instrumentation, as provided by the producer, is shown in Appendix S2.

The air was sampled simultaneously in both chambers, and both chambers received the same supply air, as mentioned earlier.



The air for the sensory assessments was delivered to a test rig through mounting slots in the side walls of the chambers, just above the floor. A flexible duct (diameter of 75 mm) attached to an axial fan was connected to the slot. The rig delivered the air from the chamber to the assessing subject outside the chamber at a height of about 1 m. Two dampers were installed in this duct. One was used to set the airflow in the duct to about 0.9 L/s; this rate is necessary to ensure that the subjects inhale only the delivered air for the sensory evaluations as has been documented in earlier studies<sup>71</sup> and used as a standard during sensory measurements. The other was capable of closing off the airflow; it was only opened when the actual sensory evaluations were taking place.

Two ducts delivered the air for sensory assessments. One of them delivered the air with the same temperature as in the chamber. The other was equipped with an exterior heating wire and delivered the air from the chamber at a temperature of 28°C; the heating effect was controlled with a transformer. The air presented for sensory assessments was heated to 28°C to separate the effect of increased temperature in the chamber on emission of bioeffluents from any effect of increased temperature on the sensory assessments; a similar approach was used by Fang et al.<sup>72,73</sup>

The area outside the chambers, where the sensory assessments took place, was ventilated and the temperature and relative humidity of the air were measured, but they were not controlled. The subjects assembled and waited for their turn to make these sensory assessments in the adjacent hall.

Sensory assessments were performed in a random order balanced across all subjects. Three scales printed on paper were used by the subjects for performing the assessments (Figure 2): an acceptability scale, an odor intensity scale, and a visual analog scale for assessing air freshness. The acceptability scale was presented first, and the other two scales were presented on a separate sheet of paper. The scale for acceptability was preceded by the following sentence: "Imagine that during your daily life in non-industrial buildings you were exposed to this air. How do you assess the acceptability of the air quality (note the dichotomy of the scale)?"

During the assessments, the subjects approached the tube delivering the air, opened the damper, took one sniff, and made their assessment immediately. They were encouraged to take another sniff when assessing another scale should they consider it necessary. If they did they were asked to take at least 3 inhalations of ambient air before inhaling the air from the duct for the second time. When they completed their assessments, they closed the damper and went back to the waiting area, where they took a break of at least 1 min before making the next assessment.

The subjects attended a practice session prior to the experiments to receive their instructions and to become acquainted with the procedures and with the use of the measuring scales. They were instructed not to drink alcohol or eat spicy food or garlic on the day prior to experiments or on the experimental day. They were additionally instructed not to use strong deodorants or perfumes.

The air for the chemical analyses was sampled through another mounting slot located on the side wall of the chambers, just above the floor; this slot was parallel to the slot used for sampling the air for sensory assessments. The air was sampled on pre-conditioned universal multisorbent tubes containing Tenax TA and activated charcoal (Markes No. C3-BAXX-5276). The weight of the sorbents was approximately 300 mg. The tube length was 89 mm and the diameter was 6.4 mm. Calibrated pumps were used for sampling. The sampled volume was 5 and 2 L and the sampling flow rate was 0.22 L/min. The air was also sampled on 2,4-dinitrophenylhydrazine (DNPH) silica cartridges; the sample volume was then 30 L. No duplicates were made. Blanks were taken. The multisorbent tubes were sent for analyses to an independent commercial laboratory (Fraunhofer-Institut für Bauphysik IB), which identified and quantified the VVOCs, VOCs, and SVOCs (up to C22) by thermal desorption gas chromatography-mass spectrometry (TD-GC/MS). Analysis was performed using a slightly polar capillary column (RTX-624) for which the retention time is mostly a function of the boiling point/molecular weight. The response factors of ethanol, isoprene, acetic acid, hexanal, 6-MHO, nonanal, decanal, and squalene were determined using existing reference standards for the GC-MS system. The concentrations of all other identified compounds were expressed as their toluene equivalent concentration by assigning the calibration curve of toluene to them, assuming that their response in GC/MS was similar to that of toluene. This assumption is acceptable for hydrocarbons without heteroatoms in the molecule in the range of C6-C16 (personal communication from the laboratory performing the chemical analyses). GC peaks  $\geq 1 \mu\text{g}/\text{m}^3$  were integrated. DNPH were analyzed for aldehydes and ketones in the range from C1 (formaldehyde) to C6 (hexanone, cyclohexanone, hexanal, methyl isobutyl ketone (MIBK)). The concentration of each of these substances was quantified individually using five-point calibration curves based on a standard solution of DNPH in acetonitrile by liquid chromatography with a diode array detector (HPLC-DAD). Only concentrations  $\geq 5 \mu\text{g}/\text{m}^3$  are reported following the recommendations of the German AgBB-scheme,<sup>74</sup> which was followed for all of the analysis. Appendix S3 provides additional details of these chemical analyses.



**FIGURE 2** Visual analog scales used for the subjective assessments: acceptability scale (left), Odor intensity scale (center), and the air freshness scale (right)

## 2.5 | Data treatment and statistical analyses

The sensory ratings made by the subjects were digitized, and the results were manually checked for transcription errors and any other gross errors. The scales were coded as follows: clearly acceptable=+1, just acceptable=+0, just not acceptable=-0, and clearly not acceptable=-1; overpowering odor=+5 and no odor=0, and fresh air=0, stuffy air=100. Measures of central tendency and variance were calculated for each condition under which sensory assessments were made.

Two-way ANOVA with a Bonferroni post hoc test was applied to detect any significant differences between conditions with bioeffluents and between conditions in the chamber, assuming that the residuals were normally distributed.

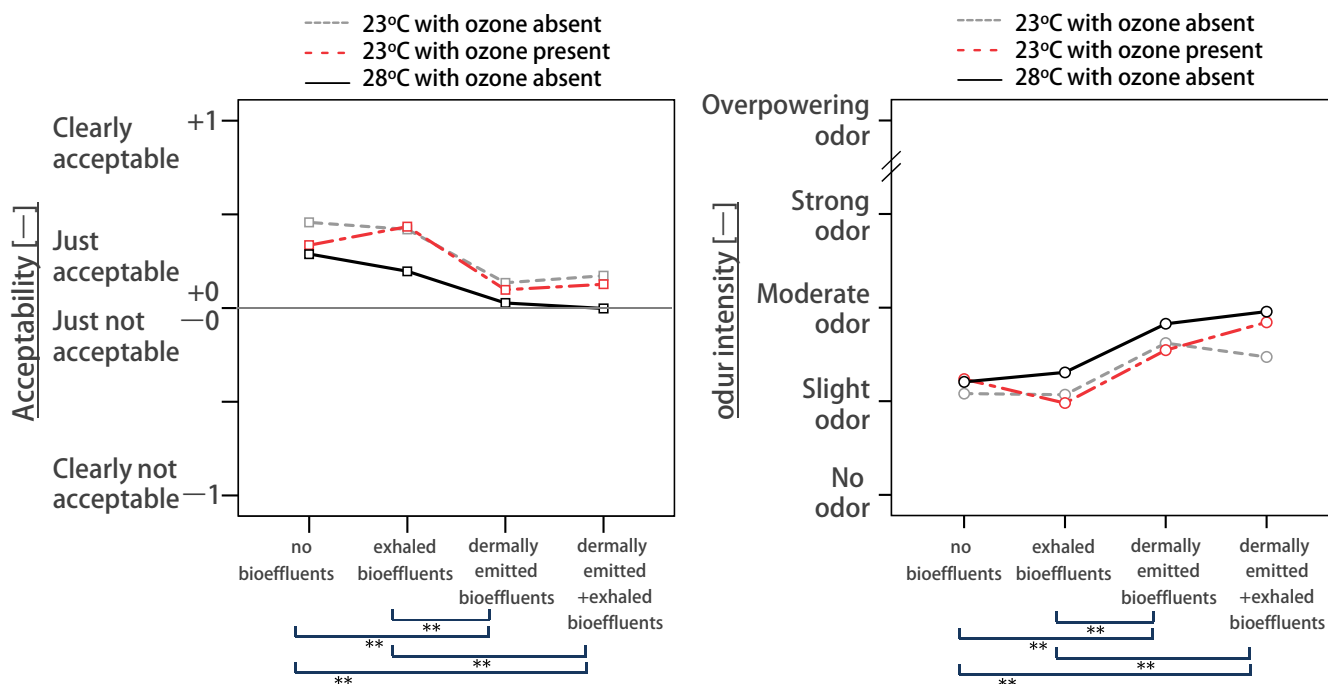
Univariate analyses were also performed using one-way ANOVA with a repeated measures design, and Fisher's least significant difference (LSD) post hoc test was used to compare pairs of sensory ratings made under different conditions.

The analyses were made with IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). In the present analysis, the significant differences were identified using *P*-values set at .01 and .05.

## 3 | RESULTS

All sensory assessments and the results of chemical analyses are shown in Appendices S4-S6. The present section reports selected results that describe the overall trends and directions identified by examining the measured data.

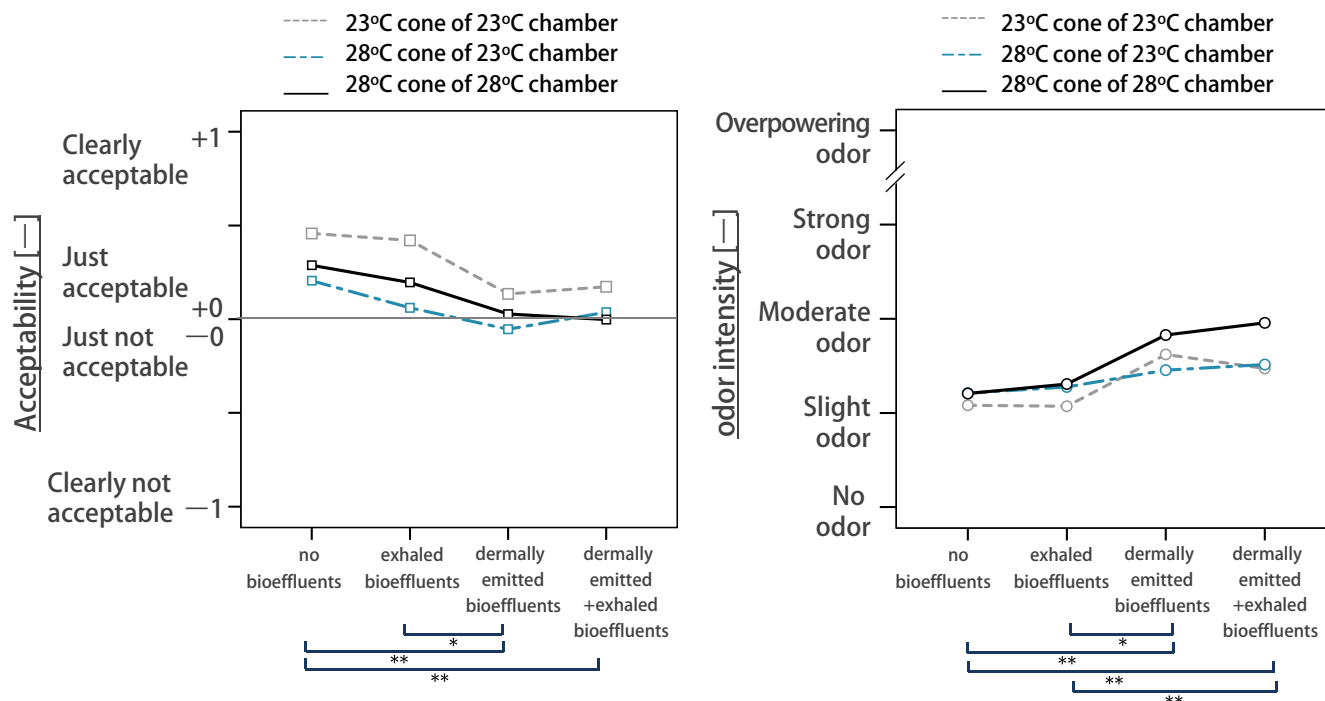
Figure 3 shows the ratings of acceptability of air quality and the ratings of odor intensity in the chambers under the different conditions examined in the experiments; the air delivered for these assessments had the same temperature as the temperature in the chamber; the results of univariate analyses are shown in the Appendix S7. Figure 3 shows that all significant differences were in the expected direction, that is, pollutants caused the air quality to be worse and the odor level to be higher. There were no statistically significant differences between sensory assessments of the air in the chamber without bioeffluents (empty) and in the chamber with exhaled bioeffluents. The sensory assessments of the air in the chamber with dermally emitted bioeffluents were significantly different from the assessments of air in the empty chamber and the chamber with exhaled bioeffluents. The sensory assessments of the air in the chamber with bioeffluents emitted by the whole body (dermally emitted and exhaled bioeffluents) did not differ significantly from the sensory assessments of air with dermally emitted bioeffluents only but were statistically significantly different from the sensory assessments of air in the chamber with exhaled bioeffluents only and from those made in the empty chamber. Figure 3 also shows that the presence or absence of ozone in the supply air did not affect the sensory assessments ( $P>.05$ ). However, when the temperature in the chamber was increased to 28°C, the sensory assessments of air in the chambers differed systematically from those at 23°C; this effect was statistically significant ( $P<.05$ ). Additional analysis was therefore made, which compared sensory ratings in the chambers with ozone absent when the temperature in the chamber was 23°C or 28°C, and the air presented for sensory assessment was 23°C or 28°C. Figure 4 shows the results of this analysis. It indicates



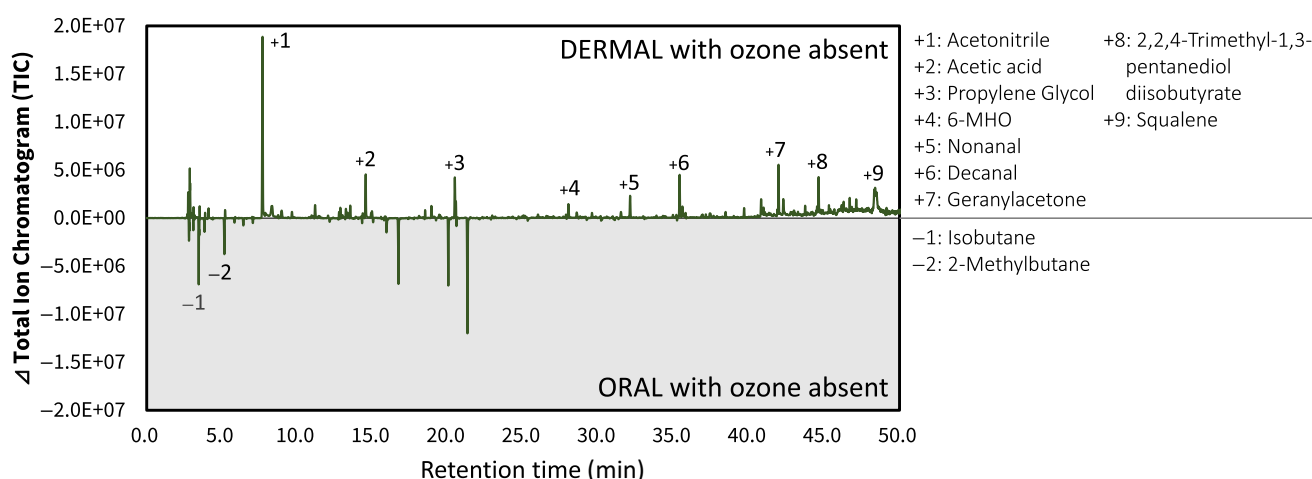
**FIGURE 3** Acceptability of air quality (left) and odor intensity (right) in the chambers at different conditions investigated in the present experiments. The air presented for sensory assessments had the same temperature as the air in the chambers. Asterisks indicate the level of statistical significance: \*\* $P<.01$

that the odor intensity of air with dermally emitted and whole-body bioeffluents increased at a temperature of 28°C; this effect was statistically significant for the whole-body bioeffluents ( $P < .05$ ). This analysis additionally shows that a higher temperature of the air presented for sensory assessments reduced acceptability but had no effect on odor intensity assessments. The assessments of air freshness (not shown here) followed trends similar to those of the ratings of acceptability.

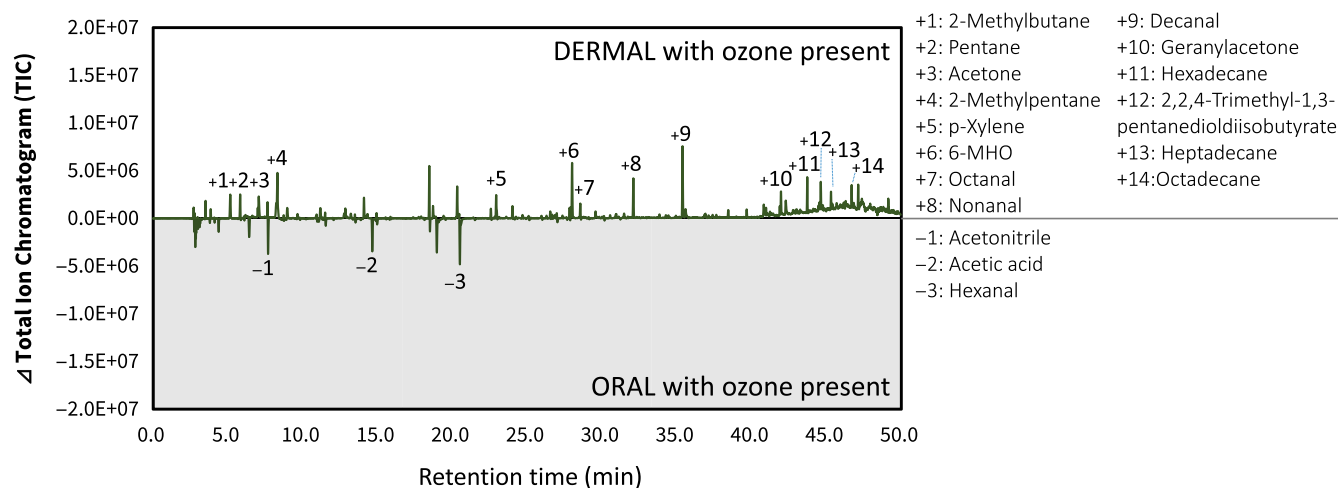
In all, 51 substances were detected by GC-MS analysis and 7 substances were detected by HPLC-DAD. Figures 5-8 show some selected results of the chemical measurements. The objective of this study was to examine the differences between dermally emitted and exhaled bioeffluents. Consequently, Figures 5-8 were generated by subtracting chromatograms for the different conditions established in the chambers. This process also corrected for the influence of pollution



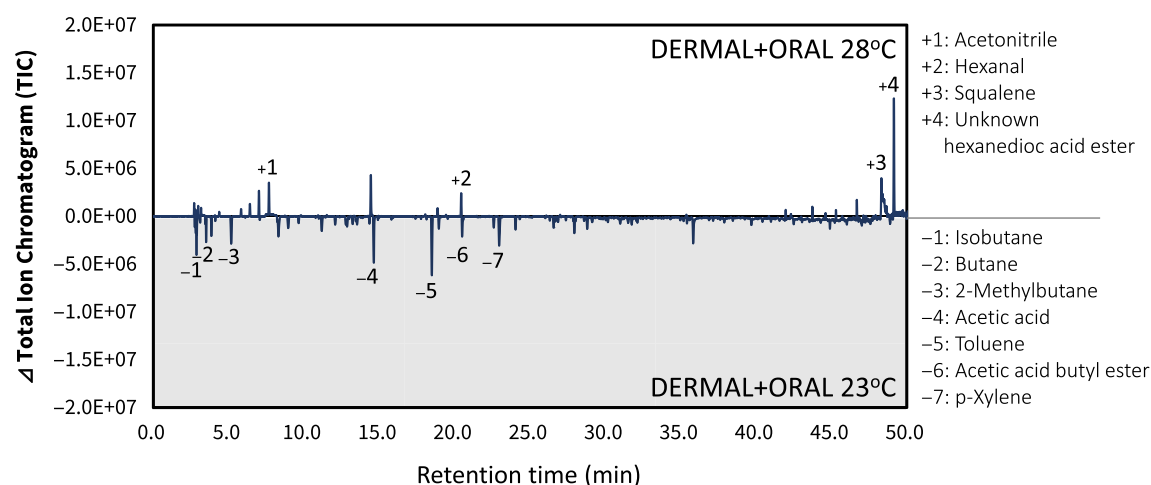
**FIGURE 4** Acceptability of air quality (left) and odor intensity (right) in the chambers at different conditions investigated in the present experiments when ozone was absent in the supply air. The air presented for sensory assessments had the same temperature as the chamber air but the air in an additional cone delivering the air from the chamber at 23°C was increased to 28°C. Asterisks indicate the level of statistical significance: \*.01 <  $P$  < .05, \*\* $P$  < .01



**FIGURE 5** A comparison between chromatograms showing the chemical composition of the air with dermally emitted and exhaled bioeffluents when the temperature in the chambers was 23°C and ozone had been eliminated from the supply air. The figure shows the result of subtraction of the chromatograms obtained for dermally emitted and exhaled bioeffluents. The positive peaks indicate dermally emitted pollutants with concentrations higher than were observed in the exhaled pollutants. The negative peaks show exhaled pollutants whose concentrations were higher than were observed in the dermally emitted pollutants. No peak or peaks close to zero indicate either that the pollutant was not present or that the concentrations observed in the exhaled and dermally emitted bioeffluents were similar



**FIGURE 6** A comparison between chromatograms of dermally emitted and exhaled bioeffluents at 23°C when ozone was not eliminated from the supply air. The figure shows the result of subtraction of chromatograms obtained for dermally emitted and exhaled bioeffluents. The positive peaks indicate dermally emitted pollutants with concentrations higher than they were in the exhaled bioeffluents. The negative peaks identify pollutants whose concentrations were higher in the exhaled bioeffluents than in the dermally emitted bioeffluents. No peak or peaks close to zero indicate either that the pollutant was not present or that the concentrations observed in the exhaled and dermally emitted emissions were similar



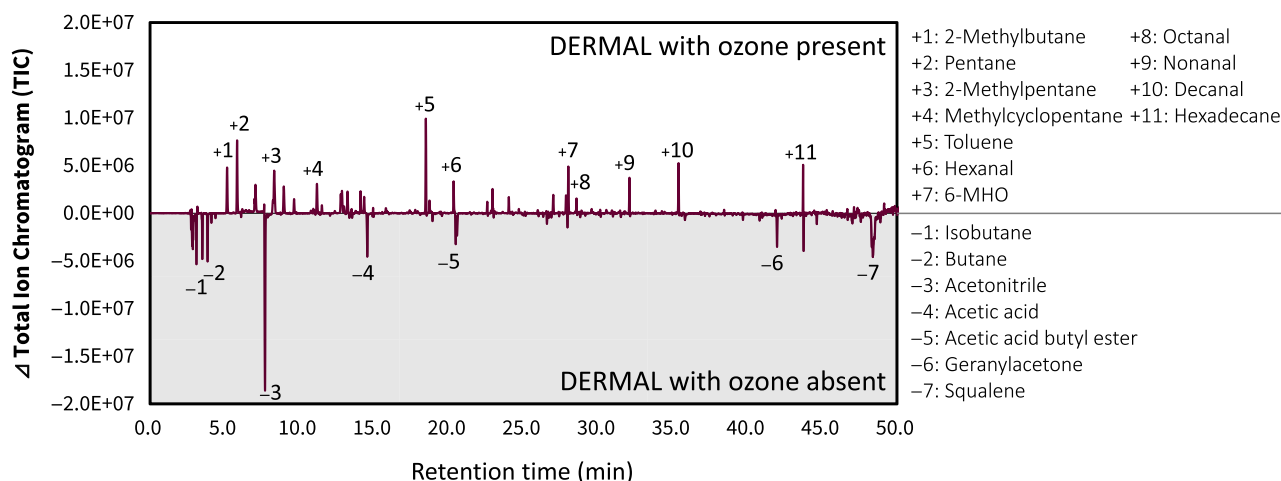
**FIGURE 7** A comparison between chromatograms of whole-body (exhaled+dermally emitted) bioeffluents at 23 and 28°C when ozone had been eliminated from the supply air. The figure shows the result of subtraction of chromatograms describing emissions at 28 and 23°C. The positive peaks indicate whole-body pollutants at 28°C with concentrations higher than were observed for whole-body pollutants at 23°C. Vice versa for the negative peaks. No peak or peaks close to zero indicate either that the pollutant was not present or that the concentrations in whole-body emissions at 23 and 28°C were similar

in the outdoor air supplied to the chambers, if any, as this parameter was also subtracted. Major peaks have been identified but a few that were not identified are left unmarked. Some of the significant peaks could not be identified. When analyzing the results, the main focus was on whether there were any differences between the conditions in terms of the compounds that have previously been associated with human bioeffluents (Table 1).

Figure 5 shows the difference in chemical composition of the air with dermally emitted and exhaled bioeffluents at 23°C when ozone had been eliminated from the supply air. The results show that greater amounts of geranylacetone, squalene, 6-MHO, nonanal, and decanal

were present in the dermally emitted bioeffluents, although less 6-MHO was present compared with geranylacetone, nonanal, and decanal.

Figure 6 shows the difference in chemical composition of the air with dermally emitted and exhaled bioeffluents at 23°C when ozone was present in the supply air. The figure shows similar but larger differences in chemical composition than in Figure 5. Squalene was not detected to the same extent and here the 6-MHO peak was higher than those of nonanal and geranylacetone. Additionally, acetic acid was identified in the exhaled air, whereas the acetone in the dermally emitted bioeffluents was probably the result of the ozone-squalene chemistry that occurred in the chamber.



**FIGURE 8** A comparison between chromatograms of dermally emitted bioeffluents when ozone either had or had not been eliminated from the supply air at 23°C. The figure shows the result of subtraction of chromatograms describing emissions with and without ozone present. The positive peaks indicate dermally emitted pollutants whose concentration was higher when ozone had not been eliminated than when ozone had been eliminated. Vice versa for the negative peaks. No peak or peaks close to zero indicate either that the pollutant was not present or that the concentrations with and without any ozone present were similar

Figure 7 shows the difference in chemical composition of the air with whole-body (dermally emitted+exhaled) bioeffluents when the chamber temperature was set at 28°C or 23°C. The results show more squalene in the chamber when the temperature was 28°C, as would be expected, and that there was more of an unknown hexanedioic acid ester at this temperature. More acetic acid was seen in the chamber when the temperature was 23°C. The other differences are unlikely to have been caused by the difference in temperatures.

Figure 8 shows the difference in chemical composition of the air with dermally emitted bioeffluents when ozone was present and when it had been eliminated from the supply air, when the temperature was set at 23°C. The results show that when ozone was present there were more substances, including some higher molecular compounds and some aldehydes. More geranylacetone and squalene were found in the chamber when ozone had been eliminated.

The concentration of the compounds detected in the different conditions was compared with their odor thresholds. This was done even though the concentrations for some compounds were obtained as toluene equivalents. It was assumed that for them they approximate the actual concentration and at least maintain the relative differences between the reported concentrations. For 38 of 59 substances detected in chemical analyses, the odor thresholds were obtained from the compilation of odor thresholds reported by Nagata,<sup>75</sup> while for 33 of 59 substances odor thresholds were found in the compilation reported by Devos et al.<sup>76</sup> The only substances for which the concentration was higher than the odor threshold in at least one of the 9 conditions with bioeffluents were decanal, hexanal, nonanal, for which the concentration was determined using reference standards, and octanal, for which the concentration was toluene equivalent. A comparison of concentrations with odor thresholds for these and other compounds is presented in detail in the Appendices S5 and S6.

## 4 | DISCUSSION

### 4.1 | Exhaled and dermally emitted bioeffluents

The purpose of this study was to examine whether dermally emitted and exhaled bioeffluents produce different sensory effects and, thus, have different contributions to the body odor problem. There was no intention in the present work to measure the emission rates or all types of bioeffluents, as the sample was small. The results show that the perception of increased odor intensity and of unacceptable air quality caused by human bioeffluents can be primarily attributed to dermally emitted bioeffluents. As shown in Figure 3, the ratings of both acceptability of air quality and odor intensity differed significantly between exposures to exhaled and dermally emitted bioeffluents. These findings are further supported by results showing that the odor intensity of air containing dermally emitted bioeffluents was similar to that of air containing whole-body bioeffluents, and that the odor intensity of exhaled bioeffluents was similar to that of air in the empty chamber. This also supports the results of the pilot experiments by the authors, which also showed that the addition of pure CO<sub>2</sub> to air containing dermally emitted bioeffluents did not significantly change the sensory ratings,<sup>77</sup> confirming the results of Zhang et al.<sup>3,4</sup> and Liu et al.<sup>13</sup>

One objective of the present research was to examine whether observed sensory effects can be partially explained by relative differences in the chemical composition of the air. Figures 5-8 show that among the compounds that were emitted dermally and from the whole body, there were aldehydes that have low odor thresholds. As acetic acid was measured among dermally emitted and exhaled bioeffluents, we assume that it did not contribute to differences in sensory perception between the two types of bioeffluents. Aldehydes with low odor thresholds had been detected in the earlier studies and are summarized in Table 1. This

suggests that the differences in sensory assessments shown in Figure 3 can be partially attributed to the presence of these compounds. Future studies should examine this hypothesis more closely, as the analytical measurements in the present experiment were limited (only toluene equivalent concentrations were measured for many compounds), as were the range and control of the ozone concentration.

It should be mentioned that halitosis can produce a local unpleasant odor due to foul breath, especially, in the immediate vicinity of the source person. However, in our experiments, the bioeffluent assessments were conducted after the exhaled bioeffluents had been mixed with the entire volume. Therefore, our results do not represent conditions under which people are close to each other. Moreover, as only organic pollutants were measured in our experiments, we were not able to determine the presence of halitosis markers; however, the odor intensity of exhaled bioeffluents was the same as that assessed in the empty chamber (Figure 3). This suggests that halitosis-causing compounds were probably at levels that could not be perceived by the subjects after being diluted in the entire volume.

The sensory evaluations of dermally emitted and whole-body bioeffluents that were made when naturally occurring ozone could enter the chambers with the supply air or when it had been eliminated were not significantly different (Figure 3). The absence of an effect of ozone could be due to the low concentration of ozone, leading to such small differences in the concentration of chemical compounds between the conditions with the ozone present or absent (Figure 8) that they could not be detected by the sensory panel. Figure 8 shows that many compounds that would be expected to evoke sensory perception were at a slightly higher concentration when ozone was present—the peaks on chromatograms were higher, but the differences were probably too small to be detected by the panel. Figure 6 shows that squalene was present at lower concentration when ozone was present, as would be expected from the reaction of ozone with squalene.e.g.<sup>66</sup> The occurrence of this chemistry is supported by elevated concentrations of the oxidation products expected from ozone/skin oil chemistry (e.g., acetone, 6-MHO, nonanal, decanal, and geranylacetone). Table 3 shows that in the conditions with ozone present in the supply air, the ozone concentration measured in the chamber was lower than in the ambient air, suggesting that ozone was being scavenged in the chamber by reactions with skin oils.

Aldehydes, geranylacetone, and 6-MHO were abundant in the dermal emissions, and they reduced PAQ and increased odor intensity. These results are in agreement with the published literature describing the processes that occur on human skin. Sebaceous glands consisting mainly of wax ester (25%), triglyceride (60%), and squalene (12%) and distributed over the entire body (except for the palms and soles of the feet) produce squalene.<sup>78</sup> Squalene has six double bonds that can easily be oxidized. This reaction produces nonanal, decanal, geranylacetone, and 6-MHO. Geranylacetone is both produced by ozone (with squalene) and consumed by ozone after it is produced. In the present study, the consumption of geranylacetone seemed to dominate, as geranylacetone was present at a lower concentration when ozone was present (Figure 8). 6-MHO also reacts with ozone once it had been formed but it apparently it was not all consumed, as may be seen in Figure 8. This is consistent

with 6-MHO being produced both by ozone reacting with squalene and by ozone reacting with geranylacetone, whereas geranylacetone is produced only by ozone reacting with squalene (and then being consumed by ozone), which is in agreement with the measurements of geranylacetone shown in Figure 8. The processes described above show that ozone and dicarbonyls react quickly in the upper layers of the skin, preventing some potentially hazardous compounds from penetrating deep into the skin and hence reaching the blood.<sup>79</sup> It is worth mentioning that sebaceous compounds mix with sweat on the epidermis and that this acid mantle has a bactericidal action on the horny cell layer (especially wax ester and squalene), promoting water retention.<sup>80,81</sup>

The absence of ozone will not completely eliminate the oxidation process, but it may reduce the rate at which squalene is oxidized. For instance, people may already have the oxidized substances on their skin as the process can occur in other places where ozone is present, for example outside the building. Geranylacetone, nonanal, and decanal have high molecular weights and hence are “sticky”; therefore, these molecules remain on the skin after they are produced. On the other hand, 6-MHO is quite soluble in skin oil and not as “sticky.” This may explain why aldehydes produced by ozone/skin oil chemistry were detected, even when ozone had been removed from the supply air, and why the concentration of 6-MHO was lower than that of geranylacetone, nonanal, and decanal (Figure 5). The concentration of 6-MHO was higher than that of nonanal and geranylacetone with ozone present in the supply air, as indicated by the higher peak (Figure 6). This is consistent with its production in the ozone/squalene reactions that occurred in the chamber when ozone was present. For completeness, it should be mentioned that the oxidation process can also be driven by other oxidative compounds such as oxygen, although this reaction is probably too slow to have had any effect in the present experiments. Future work should examine further the processes described above.

It may also be seen that there were more compounds with a retention time higher than 40 in the condition with dermally emitted bioeffluents compared with the condition with exhaled bioeffluents (Figures 5 and 6). In general, compounds with a higher molecular weight have higher retention times. The compounds exhaled tended to be more volatile, with a lower molecular weight than the compounds that were dermally emitted (Table 1 and Nicolaides<sup>82</sup>). Also, even if there had been compounds with a high molecular weight in the exhaled breath, it is likely that they would have been sorbed on the breathing mask and on the tube delivering the exhaled air to the other chamber.

There are other processes that may explain why body odor was present even when ozone was absent. Body odor is produced when the skin flora that is resident on the surface of the skin decompose the sebum and sweat through the process termed lipase action.<sup>78</sup> In particular, triglyceride is mainly hydrolyzed to fatty acid by *propionibacterium acnes* and *staphylococcus epidermidis*, even when there is no ozone, although these compounds were not detected in the present experiment. Even if the skin had been wiped, *staphylococcus epidermidis* can return to its original concentration on the skin within 30 min to 2 h. The analytical methods that were used were not capable of detecting fatty acids. Some of the undetected fatty acids may thus have been partially responsible for the observed body odor.



It should be noted that 4-oxo-pentanal (4-OPA) was not measured in these experiments. This compound of the ozone reaction with skin oil<sup>59</sup> but it is difficult to measure. The analytical limitations of our measurements may account for the lack of its detection.

2,2,4-Trimethylpentanediol diisobutyrate, a low-temperature plasticizer commonly referred to as TXIB, was detected when conditions with dermally emitted bioeffluents were compared with the condition with only exhaled bioeffluents (Figures 5 and 6), but not when whole-body bioeffluents were compared (Figures 7 and 8). TXIB should not be considered as a dermally emitted bioeffluent. It is an additive present in inks, plastisols, coatings, urethane elastomers, and nail polish lacquers and it was probably detected because the source subjects had touched something that was coated with TXIB prior to the experiments.

No duplicates were made, and the concentrations of most of the compounds measured were expressed as toluene equivalents. Hence, we only performed relative comparisons between the chromatograms (Figures 5-8).

No chemical measurements were performed on the outdoor air supplied to the chambers. However, the sensory evaluations of odor intensity in the empty chambers on different days did not indicate a difference in the quality of air supplied to the chambers. Furthermore, the quality of supplied air would not affect the comparisons presented in Figures 5 and 6, as the measurements were performed simultaneously on the same day. In the case of the results presented in Figure 7, the influence of outdoor air, if any, was reduced by the charcoal filter in the supply airflow. The negligible influence of supply air quality on the measurements is also implied by the results presented in Figure 8, which show similar results, as for the other comparisons.

The charcoal filter used to remove ozone could also remove organic compounds from the outdoor air. Whether it actually did remove the pollutants is not considered to influence the final results. Firstly, the study was performed in a rural area with a generally high air quality. Secondly, comparison of the chromatograms between the conditions with ozone present (w/o charcoal filter) and absent (w/charcoal filter) did not present significant differences in the types of pollutants measured.

Although possible impurities from the masks, the tubes connecting the masks, and the adjacent chamber were not measured, the sensory evaluations of odor intensity in the chamber containing exhaled bioeffluents did not differ from the odor intensity in the empty chamber (Figure 4), implying that no impurities were emitted from these items that evoked a sensory response.

## 4.2 | The effects of increased temperature

Although Figure 3 does not show clearly whether increased temperature increased the emission of human bioeffluents, Figure 4 shows that when the effect of temperature on perception was eliminated (the sensory ratings were made on air having the same temperature), the odor intensity of the air with whole-body bioeffluents at 28°C was significantly different from the odor intensity when the temperature was 23°C. This result implies that the emission of bioeffluents increased with increasing source chamber temperature. A similar effect can be seen in Figure 7, especially for squalene and hexanedioic acid

ester. The present results provide some support for the Australian ventilation standard<sup>83</sup> that imposes an increased ventilation requirement when ambient temperatures are higher than 27°C, to deal with the expected higher emission of bioeffluents. However, more studies are required to further investigate the effect of temperature on the emission of bioeffluents, account the recent research reported by Luo et al.<sup>84</sup> which shows that metabolic rate can be significantly influenced by ambient temperature and clothing insulation.

To explain the results observed when the temperature in the chamber was 28°C, it should be noticed that the vapor pressure of squalene is higher at 28°C than at 23°C, which means that it becomes more volatile (the melting point of squalene is -5°C so it is a liquid at indoor temperatures). Additionally, during periods with high temperatures sebum becomes soft and secretion increases, which would increase volatilization and consequently the emission of squalene. Moreover, the number of resident skin flora increases at higher temperatures; higher humidities, higher nutritional status of the skin, and pH also increase this number. Consequently, when the temperature increases, more odorous substances will be produced as resident skin flora decompose triglyceride and contribute to any sensory effects.<sup>78</sup> Finally, higher temperature will increase the rate of the oxidation reaction with squalene, according to the Arrhenius equation, which will again contribute to causing stronger sensory responses. The combined effect of higher temperature and elevated ozone concentration on the emission of bioeffluents that can reduce the PAQ and increase odor intensity warrants further attention. We recommend that this effect should be taken into account in the design of ventilation for occupied spaces.

We also recommend that the temperature of the air should be taken into account when setting ventilation requirements if the purpose is to achieve acceptable air quality. The sensory assessments of air quality performed during the present experiment confirm the previous work of Kerka and Humphreys,<sup>85</sup> Woods,<sup>86</sup> Cain et al.<sup>8</sup> Berglund and Cain,<sup>87</sup> and Fang et al.<sup>88</sup> They show that the air was perceived as less acceptable at an increased temperature and that there is either a very small or no effect of temperature on the perceived odor intensity (Figures 3 and 4).

## 4.3 | Limitations

Single replicate measurements were performed for both sensory measurements of odor intensity and chemical measurements of air composition in the chamber. This is a potential limitation of this study. However, the results were consistent across different conditions and indicate that the odor intensity of air containing dermally emitted bioeffluents was higher than that of air containing exhaled bioeffluents, and that the odor intensity in the chamber containing exhaled bioeffluents was similar to that of air in the empty chamber. These results were independent of temperature and ozone concentration changes. Furthermore, chemical measurements were consistent under different condition scenarios in the chambers and presented similar compounds for air containing dermally emitted bioeffluents under different conditions. We, therefore, conclude that the results of this study are credible.

Another limitation of the present work could be the experimental procedure. The measurements were performed after the CO<sub>2</sub> concentration reached a steady state and remained constant (see Appendix S4). The stability of CO<sub>2</sub> concentration does not guarantee that all VOCs were stable. Real-time measurements of VOCs would be needed, as reported by Tang et al<sup>18</sup> to check their stability. Additionally, on the two experimental days when ozone was being eliminated the break between different conditions was 10–30 min. and the fans supplying outdoor air were turned off in order to reduce the time needed to achieve a steady-state condition. Sensory assessments showed, however, that the impact of these procedures on the final results was negligible. For example, the background sensory assessments on the days when the outdoor air supply was not turned off at 23°C did not differ significantly from those on the days when it was turned off.

Only one “blend” of bioeffluents was examined. There could be external factors, including the diet, stress level, hygiene habits, personal care products, and very light clothing (underwear) worn by the subjects sitting in the chamber, which could have affected the emission of chemical compounds and consequently the air quality. These can be considered as limitations of the present experiment; however, it should be noted that their influence was somewhat adjusted for comparing the chromatograms at different conditions rather than looking at absolute levels. Future study of their relative influence would be a valuable addition to this field of research.

The source subjects breathed air containing dermally emitted bioeffluents when wearing the masks. This will have caused some dermally emitted bioeffluents to be drawn into the chamber together with the exhaled bioeffluents. Tracer gas measurements were performed and indicated that this effect was negligible for the overall findings.

Finally, it cannot be ruled out that other pollutants not identified by the chemical analysis carried out in the present experiments could also contribute to different sensory responses elicited by exposure to exhaled and dermally emitted human bioeffluents. Analytical challenges and limitations made it difficult to identify these compounds. It is also likely that the observed differences can be partially caused by so called “cocktail” effect, that is, the combined effect of compounds that is eliciting sensory response even when their concentrations are below odor threshold. Future experiments can provide further explanations into these matters.

#### 4.4 | Future work

Future studies should avoid the limitations described above and examine and verify some of the assumptions made in the previous sections. They should also aim to extend the present results by examining higher ozone levels than occur in Denmark, the combined impact of ozone and temperature, different concentrations of bioeffluents, different production rates of bioeffluents (by manipulating the activity level of the source subjects), and the impact of clothing, laundering of clothing, and bathing habits.

In the present study young male subjects were used as a source of bioeffluents. Other experiments must be carried out with other groups of subjects especially diversified as regards the gender (male–female)

and age (children–elderly) (e.g., Barber,<sup>89</sup> Mitro et al<sup>90</sup>) before the present results can be generalized for a broad population. Gallagher et al<sup>35</sup> reported that some compounds, such as dimethylsulphone, benzothiazole, and nonanal, were emitted from skin at a higher rate with increasing age. The number of colonies can differ from person to person depending on the composition of their skin and the composition of the sebum on the skin surface. Normally, the volume of sebum secreted peaks around the age of 10 to 20 years for females and around the age of 30 to 40 years for males. In the present experiment, the source subjects' average age was  $24.4 \pm 2.0$ , suggesting that the odor intensity would be higher if 30- to 40-year-old male source subjects had been used. Additionally, the effect of the occupants' body size, hygienic habits, and health conditions should be considered in future studies. If the source subjects had had a larger body surface area, it would also have been increased; their average height and weight were  $174.6 \pm 4.8$  and  $73.4 \pm 3.4$  in the present experiment, indicating that they were not overweight.

The observed magnitude of difference in sensory perceptions of exhaled and dermally emitted bioeffluents should not be generalized to broad population either not only because the bioeffluents were produced by young males but also because the sensory assessments were not made by the panel representing diverse groups in population with different sensory sensitivity. This also calls for additional experiments in future that would complement and generalize the present results.

## 5 | CONCLUSIONS

The presence of exhaled bioeffluents did not cause any significant change in the sensory ratings of odor intensity or the acceptability of the chamber air quality. On the other hand, the presence of dermally emitted bioeffluents (either alone or with exhaled bioeffluents) caused significant changes in the sensory ratings of both odor intensity and the acceptability of the chamber air quality, indicating that they decreased air quality.

Increasing the temperature from 23 to 28°C significantly increased the odor intensity of bioeffluents emitted by the whole body. Eliminating ozone from the supply air did not cause any change in sensory ratings of odor intensity or the acceptability of the air quality when dermally emitted, exhaled, or whole-body bioeffluents were present.

The chemical composition of air with dermally emitted or exhaled bioeffluents was different. The air with dermally emitted bioeffluents present contained aldehydes (nonanal, decanal), geranylacetone, and 6-MHO. Increasing the air temperature to 28°C increased the emission of squalene and other compounds with high molecular weight. Eliminating ozone from the supply air reduced the levels of aldehydes, geranylacetone, and 6-MHO. When ozone was not removed, the concentration of squalene was lower.

The present results are particularly relevant to the development of effective methods for improving the perceived air quality when pollutants emitted by humans are present. They indicate that dermally emitted bioeffluents may be the primary cause of any sensory effects.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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